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DEMONSTRATION BY LABELED TREPONEMAL ANTIGEN OF SPECIFIC ANTIBODIES IN THE TISSUE INFILTRATES OF SECONDARY SYPHILIS

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Specific antitreponemal antibodies have been demonstrated by immunofluorescence techniques in the lymphoplasmocytic infiltrates which characterize early syphilitic lesions. A purified suspension of Nichols strain *Treponema pallidum* was sonified and labeled with fluorescein isothiocyanate and applied to cryostat sections of 12 biopsy specimens from the cutaneous lesions of 11 patients with proven secondary syphilis, using a modified direct immunofluorescence procedure. Specimens from various inflammatory dermatoses processed similarly served as controls. Granular fluorescence was noted in the dermis in 9 of the 12 specimens corresponding to areas of heavy plasma cell infiltration and some fluorescence was found directly on plasma cells which were identified by subsequent hematoxylin and eosin staining. This fluorescence could be blocked by prior incubation of the sections with unlabeled sonified treponemal suspension. Control slides did not reveal any fluorescence. The use of labeled treponemal antigen may aid the tissue diagnosis of early syphilitic lesions which can mimic a variety of dermatological disorders.

Human syphilitic infection is associated with a complex antibody response against treponemal and certain nontreponemal antigens; among the latter, mitochondrial membrane cardiolipin material ordinarily extracted from beef heart has received most attention. In early syphilis these antibodies are detectable in high titers in the patients' sera [1-3]. In nephropathy associated with secondary syphilis and the nephrotic syndrome in congenital syphilis, where very high titers of such antibodies occur, electron microscopic and immunofluorescence studies of renal biopsy specimens have revealed subepithelial deposition of electron-dense material and immune complexes along the glomerular basement membrane [4-12].

Early syphilitic lesions feature prominent accumulation of lymphoplasmocytic infiltrates which are predominantly perivascular [1,13,14]. Such lesions can mimic a variety of dermatoses both clinically and histologically. Clinical resemblance to papulosquamous diseases and also histological similarities to disorders such as psoriasis, lichen planus, lichenoid drug eruptions, erythema perstans, and granulomas are well known [13-15]. The histological diagnosis of early syphilitic lesions would be much aided if antitreponemal antibodies could be demonstrated in the tissue infiltrates.

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Abbreviation:
FITC: fluorescein isothiocyanate

The present study was designed to develop a method for the detection of such specific antibodies in the biopsy sections of cutaneous lesions in patients with secondary syphilis.

MATERIALS AND METHODS

Tissue Specimens

Twelve biopsy specimens were obtained with a 4-mm Keyes punch from cutaneous papular lesions of 11 patients with secondary syphilis proven by serological tests and darkfield microscopic examination [16]. Since the lesions were dark-field positive, tissue sections were not stained with silver. The biopsy specimens were immediately frozen in liquid nitrogen. Six micron thick cryostat sections were cut from these specimens and stored at −20°C until processed. Biopsy specimens from normal skin and lesions of pityriasis rosea, urticaria pigmentosa, chronic eczematous dermatitis, lichen planus, psoriasis vulgaris, and chronic gingivitis were similarly obtained, sectioned, and stored.

Treponemal Antigen

Lyophilized and electrophoretically purified Nichols strain of *T.* pallidum, routinely used for the FTA-ABS test, was purchased from Beckman Instruments, Inc. Each vial contained approximately 10 mg of lyophilized treponemes. The contents of four vials were reconstituted with 3 ml of distilled water and sonified for six minutes with a model W 185 Sonifier Cell Disrupter (Ultrasonics Inc.) using the microtip attachment at 30-40 w power. Dark-field examination revealed that over 90% of the sonified treponemes were disrupted into small fragments ranging in size up to 1 or 2 spirals. The sonified preparation was labeled with fluorescein isothiocyanate (FITC) using an original F/P ratio of approximately 15. Six mg of a 10% FITC powder in 1.5 ml of 0.1 M Na_2HPO_4 solution, pH 9, was slowly added to 0.75 ml of 0.2 M Na_2HPO_4 solution containing 3 ml of sonified treponemes. The pH was then adjusted to 9.5

with 0.1 M Na_3PO_4. The final mixture was gently stirred and allowed to sit for 2½ hours at room temperature. The precipitate was removed by centrifugation. The solution was then placed in Spectropor dialysis tubing with a molecular weight cutoff at about 3500 and dialyzed against 1000 ml of phosphate-buffered saline solution, pH 7.6, to remove unconjugated FITC. The dialysate was changed daily for six days until no free FITC was detected by Wood's lamp, long wave ultraviolet fluorescence. The entire procedure was carried out at 4°C. Precipitate was removed by centrifugation and 0.05 ml of a 1/1000 thimerosal solution (N.F.) was added as preservative. The final preparation was divided into small aliquots and stored at −80°C until used.

Immunofluorescence Procedures

The labeled treponemal preparation was initially absorbed with chopped normal skin, diluted serially, and used to stain 6 μ-thick frozen sections of normal skin biopsy specimens using routine immunofluorescence procedures [17,18]. The specimens were examined with a fluorescence microscope to determine the optimal dilution of each labeled sonified treponemal preparation by eliminating the undesired nonspecific fluorescence which occurred at higher concentrations. The determined optimal dilution of the labeled sonified treponemal preparation (1/10) was used on the frozen sections of the biopsy specimens obtained from the secondary syphilitic skin lesions. These sections were stained with the labeled, sonified treponemal antigen by direct immunofluorescence technique [17,18]. The preparations were mounted in elvanol and sealed with nail polish [19]. The specimens were examined with a fluorescence microscope with a 200 w mercury arc lamp, exciter filter 702 and barrier filter OG-1, and photographed. Unmounted duplicates and unprocessed sections were stained with hematoxylin and eosin for light microscopic examination. Frozen sections from the lesions of pityriasis rosea, chronic eczematous dermatitis, urticaria pigmentosa, lichen planus, psoriasis vulgaris, and chronic gingivitis, similarly processed with the 1/10 dilution of labeled treponemal antigen, served as controls.

In an additional experiment the antigenic activity of the labeled treponemal preparation was assessed by incubating sections from syphilitic lesions with unlabeled sonified treponemal antigen prepared as above but undiluted prior to the above procedures.

RESULTS

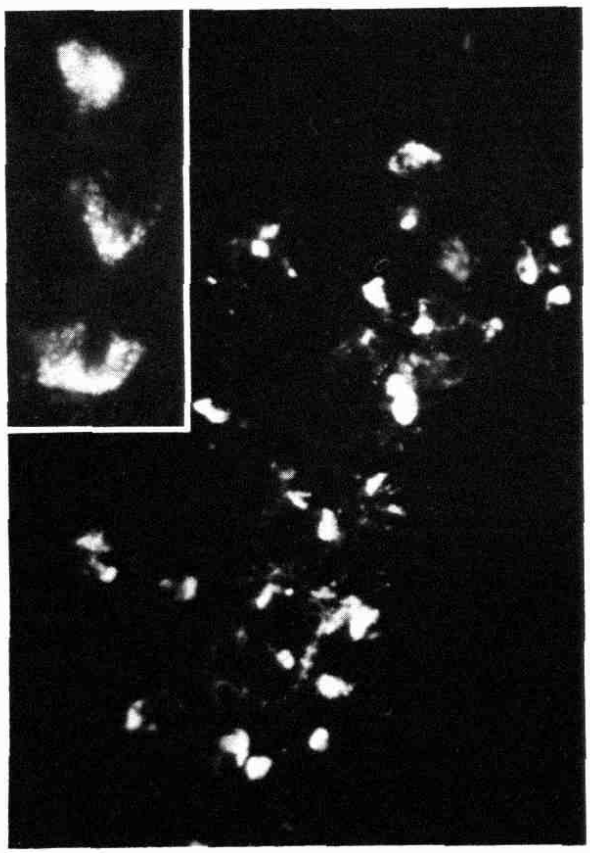
Nine of the 12 specimens revealed granular patterns of fluorescence in the dermis which on high magnification appeared to be located on cells (Figure). Light microscopic examination of the hematoxylin and eosin preparations from adjacent sections of the lesions with classic lichenoid and perivascular infiltrates showed that the fluorescence findings correlated to areas containing many plasma cells and some fluorescence was directly located on the plasma cells. Of the two biopsy specimens obtained from one patient only the one with the dense dermal inflammatory infiltrate showed fluorescence, while the specimen with a mild infiltrate did not reveal detectable binding of the labeled antigen. There was no fluorescence detectable in the epidermis or at the dermal-epidermal junction. Control slides were all negative. Preincubation of the sections with unlabeled sonified treponemal antigen blocked the binding of labeled antigen in the tissue sections.

DISCUSSION

The clinical histological findings in early syphilitic lesions, although usually quite suggestive of the disease, are by no means pathognomonic [1–3, 13–15]. Dark-field examination of the lesions and serological studies have remained the main basis for diagnosis in the early stages of syphilis [1,16, 20,21]. The more recent serological tests are sufficiently sensitive and specific for the diagnosis of the disease [1,20–23].

The diagnosis of papulosquamous eruptions, however, remains problematic in patients with highly reactive serological tests who give a history of recent syphilitic infection with questionably adequate therapy. Immunopathological findings in such lesions may prove helpful. Such techniques may also aid the diagnosis of dermatologic disorders coexisting with syphilis. The sensitivity and specificity of the technique would have to be assessed in a large number of specimens including lesions with few or no plasma cells before its possible practical clinical usefulness can be determined.

Early syphilitic lesions usually feature promi-



Demonstration of positive fluorescence with labeled antigen in papular skin lesion of secondary syphilis (reduced from × 400). *Insert* is a higher magnification (reduced from × 1000) showing the granular pattern of fluorescence on the infiltrating inflammatory cells.

nent plasma cell accumulation [1, 13–15]. Demonstration of specific antitreponemal antibodies on the plasma cells suggests that such cells may be engaged in the production of the antibodies. The specificity of the immune complexes deposited subepithelially along the glomerular basement membrane in patients with syphilitic nephropathies might also be assessed by the use of labeled treponemal antigen.

This fluorescence technique using labeled antigen might also have applicability in the immunohistological diagnosis of other disorders which feature specific plasma cell infiltrates such as deep mycoses [13,14].

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